

## **ENCLOSURE II**

### **ENVIRONMENTAL CONTAMINANTS STUDY DESIGN**

## STUDY PROPOSAL

**STUDY TITLE:** Endocrine disrupting chemicals and reproductive impairment in the Florida panther.

**STUDY OBJECTIVE(S):**

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- gather baseline data regarding hormone activity, contaminant levels and immune response in female Texas cougars prior to release into panther habitat
- determine normal hormone concentrations in both male and female adult panthers
- determine contaminant impacts on offspring of introduced female Texas cougars

**BACKGROUND/JUSTIFICATION:** The Florida panther (*Felis concolor coryi*; panther) population hangs precipitously near the edge of extinction. Only 30-50 free-ranging animals remain; primarily in the Big Cypress/Fakahatchee Strand area of southwest Florida. Several problems are prevalent in the population: most males are cryptorchid; sperm count is low; ejaculate volume is low; sperm motility is low; most spermatozoa are abnormal; several animals suffer from hyperthyroidism; and immune system impairment seems evident. It has long been the opinion of many involved that these problems are all traceable to inbreeding. However, most of the world's large cats are inbred and some of these symptoms (e.g., cryptorchidism) are not observed in other populations, or, if observed, are not of the same magnitude. At any rate, it has been proposed that the introduction of genetic diversity in the form of six to eight female Texas cougars (*F. c. stanleyana*; cougar) should improve the overall situation.

Preliminary investigation has shown that: 1) the panther is on the verge of extinction, with only 30 to 50 animals remaining; 2) the remaining panther habitat has been impacted by agricultural chemicals, many of which have been shown to disrupt the endocrine system; 3) the Florida panther is dependent upon an aquatic-based food chain with the raccoon as the major vector for exposure; 4) the panther has been exposed to a variety of potential endocrine-disrupting chemicals (some animals contain high levels of *p,p*-DDE and PCBs in their body fat); 5) the population is affected by a suite of symptoms which, taken as a whole, appear to be unique to the panther; and, 6) all of the symptoms expressed by members of the population have been shown to result, in other species, from exposure to one or more of the chemicals present within the ecosystem. Thus, we believe that an alternative explanation for the maladies exhibited by the panther exists in the form of environmental contaminants.

## PROCEDURE:

A. **Methods and Materials:** Several groups of animals will be utilized during the course of this study. These include, but are not limited to:

- cougars to be introduced into the panther population;
- captive cougars;
- free-ranging cougars;
- captive panthers;
- free-ranging panthers;
- zoo animals;
- cougars from reference populations in West Texas/Northern Mexico, Bolivia/Paraguay and Central America (Belize);
- offspring of introduced cougars;
- offspring of free-ranging panthers; and
- captive-bred offspring.

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The following tasks are to be accomplished (the order in which they are presented does not necessarily imply relative priority for the task):

- all available archived sera, and sera from those groups or populations noted above will be analyzed for hormone activity; the specific hormones to be analyzed are estradiol-17 $\beta$ , total estrogens, testosterone, progesterone (females only), T<sub>3</sub>, and T<sub>4</sub>. Sera will be collected from offspring at ages 6-8 months and 18-20 months; prior to reaching sexual maturity.
- whole blood from all animals noted above will be analyzed for mercury.
- lipid samples currently archived will be analyzed for organochlorine pesticides, endosulfan and polychlorinated biphenyls; muscle tissue biopsies from all adult females will be analyzed for these same analytes.
- histopathological examination of archived gonadal tissues from Florida panthers will be performed to determine whether any exogenous-estrogen-induced abnormalities may exist.
- immune response will be ascertained for all animals by utilizing three monitoring methods: 1) blood cell analyses; 2) mitogen stimulation procedures; and, 3) immune responsiveness to a viral vaccine.
- tissue biopsies will be collected annually from introduced female cougars and analyzed to determine build-up of contaminant load.
- sperm will be collected via electroejaculation from male offspring of introduced cougars at age 24-36 months.
- blood samples will be collected monthly for 12 months from captive females to determine the range of normal hormone levels during the annual cycle.

**Results:** Results of this study will be prepared for publication in one or more refereed journals.

## **ROLES AND RESPONSIBILITIES:**

### **Principal Investigators:**

Dr. Charles F. Facemire, U.S. Fish and Wildlife Service, Atlanta, Georgia  
Dr. Michael R. Dunbar, Florida Game and Fresh Water Fish Commission, Gainesville, Florida  
Dr. Timothy S. Gross, BEECS Program, University of Florida, Gainesville, Florida

It is anticipated that the tasks outlined above will be accomplished by the following persons or organizations:

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| ● immunology                | BEECS Laboratory, University of Florida            |
| ● mercury analyses          | U.S. Fish and Wildlife Service contract laboratory |
| ● hormone radioimmunoassays | BEECS Laboratory, University of Florida            |
| ● histopathology            | BEECS Laboratory, University of Florida            |
| ● organic analyses          | Mississippi State University analytical laboratory |
| ● wild animal capture       | Roy McBride  |
| ● sample collection         | Roy McBride/Mike Dunbar, Florida GFWFC             |

**SCHEDULE:** It is anticipated that the study will require 3-5 years (3 years for an initial investigation as outlined above; two additional years based on outcome). The study should be initiated prior to the release of the female cougars, or with sampling of offspring captured during the regular capture season in November 1994, depending on which event first transpires. Timing of future events will be dependent upon sampling opportunities.

**OPERATIONAL COST ESTIMATE:**

Supplies (\$1,500/yr x 3 yrs)	\$ 4,500
Other:	
Animal capture, sample collection, handling and shipping	
(West Texas/Northern Mexico - 10 adult males: \$ 4,000)	
(Bolivia/Paraguay - 10 adults: \$ 11,000)	
(Central America - 10 adults: \$ 9,000)	\$ 24,000
Graduate Student (University of Florida; \$20,000/yr x 3 yr)	\$ 60,000
Lab Technician (Florida GFWFC laboratory; \$18,000/yr x 3 yr)	\$ 54,000
Travel and other associated costs	\$ 9,000
<b>Total Operational Cost</b>	<b>\$151,500</b>

**DRAFT****ANALYTICAL COST ESTIMATE:**

Inorganic (mercury) analyses (\$50 ea x 100 samples)	\$ 5,000
Organic (OCs, PCBs) analyses (\$700 ea x 85 samples)	\$ 59,500
Radioimmunoassays (\$37 ea x 200 samples)	\$ 7,400
Immunology (validation of methodology, testing of all animals)	\$ 15,000
Histopathology (\$25 ea x 30 samples)	\$ 750
<b>Total Analytical</b>	<b>\$ 87,650</b>

**GRAND TOTAL (for first 3 years)** **\$239,150**